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Original contribution

Direct analysis of genetic variability in *Trypanosoma cruzi* populations from tissues of Colombian chagasic patients☆

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Summary The clinical symptoms of Chagas disease are highly variable and are correlated with geographical distribution and parasite genetic group. *Trypanosoma cruzi* group I is associated with chagasic cardiomyopathy in Colombia and other countries in northern South America. However, in southern South America, *T cruzi* group II predominates and is associated with cardiomyopathy and digestive forms of the disease. The aim of this work was to determine the correlation between the genetic profiles of *T cruzi* groups circulating in the biological cycle and those present in tissues from patients with Chagas disease. We genotyped *T cruzi* in 10 heart tissue samples from patients with cardiomyopathy from a highly endemic area of Colombia. The genotyping was performed using nuclear and mitochondrial genes and low-stringency single-specific primer polymerase chain reaction. As expected, the predominant genetic group was *T cruzi* group I; however, we also detected *T cruzi* group II. Microsatellite analyses suggested a predominance of monoclonal populations, and sequence alignments showed similarities with Colombian strains. In addition, kinetoplast DNA signatures obtained by low-stringency single-specific primer polymerase chain reaction allowed us to group strains into the 2 genetic groups. Thus, we conclude that both *T cruzi* genetic groups are producing severe cases of Chagas disease in Colombia. We did not observe any correlation between low-stringency single-specific primer polymerase chain reaction profiles, histopathologic findings, clinical forms, and severity of Chagas disease.

1. Introduction

Chagas disease (CD), a complex zoonosis caused by the protozoan parasite *Trypanosoma cruzi*, is an important public health problem in South and Central America. In Colombia, an estimated 1.3 million people are infected with the parasite, and 3.6 million are at risk of acquiring the infection [1]. Santander province presents high seroprevalence rates, reaching 40% in some localities [2]. Studies of the genetic variability of *T cruzi* populations have shown a high degree of polymorphism, and at least 2 genetic groups, named *T cruzi* I (TcI) and *T cruzi* II (TcII), were initially described [3]. Recently, an expert committee has met for the revision and standardization of the nomenclature of *T cruzi*

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Table 1 Histologic findings in analyzed tissues

Patient (sample)	Organ	Amastigote nests	Fiber aggression	Lymphocyte infiltrate	Focal fibrosis	Diffuse hypertrophy	General description
1 (B-01)	Heart	Scarce (+)	Marked (+++)	Severe (++++)	Absent	Absent	Severe inflammation and necrosis
2 (B-02)	Heart	Scarce (+)	Marked (+++)	Severe (++++)	Absent	Absent	Severe inflammation and necrosis
3 (B-03)	Heart	Moderate (++)	Moderate (++)	Marked (+++)	Moderate (++)	Scarce (+)	Moderate inflammation
4 (B-07)	Heart	Severe (++++)	Severe (++++)	Severe (++++)	Absent	Absent	Severe inflammation and necrosis
5 (B-08)	Heart	Absent	Absent	Absent	Scarce (+)	Absent	No inflammation, discrete fibrosis
6 (B-09)	Heart	Severe (++++)	Severe (++++)	Severe (++++)	Absent	Absent	Severe inflammation and necrosis
7 (B-11)	Heart	Absent	Scarce (+)	Scarce (+)	Moderate (++)	Moderate (++)	Fibrosis with mild inflammation
8 (B-16)	Heart	Absent	Scarce (+)	Scarce (+)	Scarce (+)	Scarce (+)	Fibrosis with mild inflammation
9 (B-32)	Heart	Absent	Scarce (+)	Scarce (+)	Scarce (+)	Absent	Fibrosis with mild inflammation
10 (B-34)	Heart	Scarce (+)	Marked (+++)	Severe (++++)	Absent	Absent	Severe inflammation and necrosis

strains. By consensus, the committee recognized that the defined parasite genotypes fall into 6 discrete typing units, now designated as *T cruzi* I-VI [4]. In this work, the new nomenclature is used, but the correspondence to the former subgroup denominations proposed by Brisse et al [5] is also indicated. The present TcI and TcII discrete typing units correspond to the 2 original major groups described above. In Colombia, a predominance of *T cruzi* group I has been reported in wild reservoirs, vectors, and humans [6,7]. Most of the isolated strains belong to the Z1 zymodeme (TcI), whereas Z3 and Z2 (TcII) parasites represent a small percentage and have only been isolated from vectors or wild reservoirs [6,7]. However, these isolates were obtained after in vitro culturing of blood samples and intestinal contents from triatomine bugs, which favors the selection of more highly adapted clones.

In countries such as Brazil, Argentina, and Chile, TcII has been associated with the domestic cycle and severe chronic forms of the disease, including digestive manifestations, whereas TcI has been correlated with the sylvatic cycle and a lower pathogenic potential [8]. In contrast, in Colombia, Venezuela, and Central America, heart failure and other cardiac complications are the most frequent manifestation of the disease, and the digestive mega-syndrome is apparently absent [9,10]. This clinical heterogeneity could be influenced by the genetic variability of *T cruzi* populations; however, previous studies have been unsuccessful in correlating specific types of genetic variability with clinical characteristics of the disease and differential tropism to host tissues [11,12]. This result could be explained by the vertebrate host acting as a biological filter via the action of the immune system or by clonal selection of a subpopulation from a natural mixture when parasites are grown in the laboratory. Therefore, parasites obtained from patients' blood and cultured in vitro under laboratory conditions could represent only a fraction of circulating parasites [13]. Different clones from the same strain could also have different tropisms toward different tissues, which means that parasites circulating in the blood could differ from those present in tissues. Thus, to determine the genetic group responsible for a given pathology, it is necessary to genotype the parasites present in affected tissues. The low-stringency single-specific primer polymerase chain reaction (LSSP-PCR) technique enables the genetic profiling of *T cruzi* kinetoplast DNA (kDNA) minicircles from clinical specimens including heart tissues, esophageal tissues, and blood from patients in the chronic phases of CD [14,15]. Recently, we described for the first time the direct detection of TcII in blood samples from Colombian patients using PCR technique, although TcI remains predominant in this region [16]. However, there have been no studies aimed at genetically characterizing groups of *T cruzi* directly from affected tissues of chagasic patients from Colombia or other countries of northern South America or Central America. For this reason, in this study, we genotyped *T cruzi* present in heart tissue from patients with chagasic cardiomyopathy using nuclear and mitochondrial genes. In addition, we genotyped isolates obtained from the peripheral blood of patient and from vector in the highly endemic area of Santander province, Colombia. We used the LSSP-PCR technique to detect a correlation between the genetic profile of the *T cruzi* group circulating in the biological cycle and that of the group directly responsible for the clinical form and severity of the disease.

2. Materials and methods

2.1. Tissue samples

Unfixed and formalin-fixed paraffin-embedded tissues from necropsies of individuals from the endemic area of Santander (Colombia) were collected at the Pathology Department of the Universidad Industrial de Santander and at the Instituto Nacional de Medicina Legal y Ciencias Forenses. These samples comprised 327 heart tissue samples with histopathologic alterations such as dilated cardiopathy, congestion, and focal fibrosis. Endemic areas for CD in the Department of Santander are mainly rural, so diagnosis is not easy, and autopsies are not possible to determine the cause of death. Therefore, the samples used in this study do not constitute a systematic study but are tissues collected at the institutions listed above that showed pathologic features of chagasic dilated cardiomyopathy. As a control, heart samples